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Purification of Inositol from Plant Materials

Field of the Invention

This invention relates to production of inositol from plant materials.

Background to the Invention

Inositol is a highly valued B-vitamin. Plants contain phytic acid {myoinositol 1,2,3,4,5,6-hexakis (dihydrogen phosphoric acid)} as the storage form of phosphorus. Phytic acid is found within plant cell structures as mineral bound complexes termed phytin. Phytin is largely insoluble at neutral pH. Phytic acid can also exist in solution in the salt form termed phytate. The terms "phytin" and phytate are often used interchangeably. In this disclosure, the term "phytate" is intended to refer to phytic acid, phytate and phytin, except where a distinction between these materials is made specifically.

Some of the partial hydrolysis products of phytate are inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), inositol triphosphate (IP3), inositol diphosphate (IP2) and inositol monophosphate (IP1). These partial hydrolysis products of phytate can be hydrolyzed further to yield inositol. The obtaining of inositol from a plant material requires conversion of the phytate to inositol and purification of the inositol from other components in the plant starting material.

Producing inositol from plant material is difficult. One approach is to hydrolyze the phytate in an aqueous slurry, to yield various sugars including inositol. However, inositol is a neutral soluble sugar that is very similar in molecular size and charge

characteristics to other sugars such as glucose that are often present in high levels in plant materials. Because of this, it can be difficult to separate the inositol from the other carbohydrates in the slurry.

Another approach to production of inositol from plant materials is to purify the phytate from the starting slurry and to hydrolyze the purified phytate to inositol in a later step in the overall process. However, because phytate in plants usually exists in the form of phytin, direct phytate purification from an aqueous slurry of plant materials requires solubilization of phytin and then separation of the phytate from the remainder of the components of the slurry. Efficient extraction, solubilization of phytin and separation from the remaining components of the slurry is difficult.

Description of the Invention

This invention describes a useful and novel process for overcoming the inherent difficulties in obtaining inositol from plant materials.

In accordance with the inventive process, phytate in an aqueous slurry of plant material is partially hydrolyzed by incubating the slurry with an enzyme product enriched in phytase. The soluble fraction of the slurry is separated into anionic and neutral fractions. The anionic fraction is then hydrolyzed further, and is in turn separated into ionic and neutral fractions.

The neutral fraction thus obtained is rich in inositol, and does not contain significant quantities of other sugars which would be hard to separate from it.

Description of the Drawings

Figure 1 is a process flow chart depicting processing stages in accordance with the invention.

Detailed Description of the Invention

According to the invention, an aqueous slurry of plant material is partially hydrolyzed using phytase enzyme. Figure 1 shows a process flow chart of the various steps in the process of the invention.

As shown in Figure 1, an aqueous slurry of plant material A is subjected to partial hydrolysis with phytase. This is preferably done by incubating the aqueous slurry with phytase, at suitable temperature and pH to encourage partial hydrolysis.

The phytase enzyme can hydrolyze phytate to inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), inositol triphosphate (IP3) and inositol diphosphate (IP2). However, the phytase has little activity for hydrolysis of inositol 2-monophosphate inositol (IP1). Acid phosphatase can readily hydrolyze IP1 to free inositol, which is not desired at this point in the inventive process. Thus, the source of phytase used preferably contains little or no acid phosphatase. A source of phytase containing acid phosphatase activity can also be used, if reaction conditions are chosen to favour phytase activity and avoid substantial hydrolysis of IP1 by acid phosphatase. In using a source of phytase containing acid phosphatase the preferred

pH of the reaction is greater than 3.0 and less than 7 for optimum phytase activity without substantial hydrolysis of IP1 to inositol.

IP5, IP4, IP3, IP4, IP2 and IP1 are the major products of the reaction. They are highly soluble negatively charged compounds that exist in solution in the partially hydrolyzed slurry (shown as "B" in Figure 1). The partially hydrolyzed slurry is separated by physical separation means, such as filtration or centrifugation, to generate an inositol phosphate-containing soluble fraction (a "total soluble" fraction called C in Figure 1,) and an insoluble fraction (called "D" in Figure 1).

Unlike inositol, inositol phosphates have a negative charge. It is therefore possible to separate the total soluble fraction into an anionic fraction and a first neutral fraction, with the inositol phosphates passing into the anionic fraction. Depending on how the separation is carried out, any cationic soluble materials present may remain either with the anionic fraction or the first neutral fraction.

Total soluble fraction C is therefore separated into a first ionic fraction enriched in anionic constituents – called "ionic fraction 1" or "E" in Figure1) and a first fraction enriched in neutral constituents (and possibly cationic constituents as well, called "F" in Figure 1). Ionic fraction 1 (E) contains most of the inositol phosphates, and the neutral fraction contains most of the neutral soluble constituents of the total soluble fraction. The separation is done using known techniques for the separation of charged ionic species from soluble neutral compounds in a solution. Such techniques are, for example, ion exchange, ion exclusion, or ion retardation column separations. If it is desired to retain the cationic components in the neutral fraction, a cationic ion

exchange resin can be used, which will separate out only the anionic components into the first ionic fraction. If it is desired that the cationic components are separated out as well, then mixed anionic and cationic exchange resins can be used. The important thing at this stage is to end with one fraction which contains the anionic components and a second which contains the neutral components. Cationic components are not of concern in the process of the invention, so they can remain in either fraction.

The next step in the process is to complete the hydrolysis of inositol phosphates in the ionic fraction. This process can be done with enzymes such as phytase or acid phosphatase or without enzyme-based catalysis under controlled conditions of temperature, pressure and pH. Suitable conditions for inositol phosphate hydrolysis are known, and can be chosen according to the particular reaction equipment available. The preferred approach is to use an enzyme source containing acid phosphatase at a pH of less than 4 for optimum activity. Complete hydrolysis of inositol phosphates will generate an anionic fraction (G in Figure 1) which contains various anionic compounds from fraction E as well as neutral inositol.

Inositol can be separated from the remainder of the soluble compounds in the anionic fraction G using known techniques for separating charged from neutral compounds in solution, such as, for example, an ion exchange, ion exclusion or ion retardation column. This process generates a second ionic fraction (–called herein ionic fraction 2, and indicated in Figure 1 as "H'). and a second neutral I fraction ("I" in Figure1) rich in inositol. The inositol in the second neutral fraction can then be concentrated, crystallized and dried to form a final dry purified inositol product.

The invention has been described by reference to preferred embodiments, but it will be understood that other embodiments will be evident to a person skilled in the art. It is therefore desired that the invention shall not be limited by the particular embodiments shown, but shall include such other embodiments as would occur to a skilled person.